Innovations

Breeding the bottom line

Maxygen Inc.

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What expert breeders have done for dogs and apple trees, the biotech company Maxygen Inc. is doing for commercially important molecules — breeding them to come up with better alternatives. The technique is straightforward, says Maxygen's Dutch-born chief scientist and co-founder Pim Stemmer, upon whose work the company's technology is based. "Even a child can breed dogs or vegetables — the trick is to just look at the appearance and select for it," says Stemmer.

It is a trick that has led to two recent jackpots for Maxygen (Redwood City, California). In March, DSM Anti-Infectives of Delft, the Netherlands, paid an undisclosed amount to Maxygen to use its technique to rapidly evolve enzymes to manufacture antibiotics. And in January, Maxygen signed a deal for a potential $85 million to apply its evolution-while-you-wait regimen to food plants like corn on behalf of Pioneer Hi-Bred International Inc. of Des Moines, Iowa (later swallowed by DuPont), the world's largest producer of seed corn.

A charmed approach

Maxygen's technique of gene shuffling, as molecular breeding is also known, is "charmed," says Laura Landweber, a professor of molecular biology at Princeton University in Princeton, New Jersey, because it "starts from a mixture of nature's best attempts" and then seeks to improve them still further. Landweber herself investigates the way lower organisms like ciliates shuffle their own genes during reproduction. Maxygen's version of the technique, she says, "captures the very forces of evolution that give rise to life itself — the combinatorial forces that first put together functional genes."

The molecular breeding approach parts company with the premise of the first wave of 'evolutionary biotechnology' companies in the early 1990s. Then, academics-turned-biotechnologists attempted to evolve catalysts and other functional molecules out of RNA. The problem there, says Landweber, was that many of these approaches "ran up against the brick wall of trying to convince RNA to catalyze reactions other than [those for which RNA catalysts had originally evolved]".

By breeding molecules like cattle, Maxygen is improving detergents and drugs.

By taking on the ostensibly more difficult task of applying multiple rounds of mutation and selection on noncoding molecules like proteins, Maxygen has succeeded where many of its predecessors have not.

Irrational drug design

The technique of gene shuffling had its roots in Stemmer's early frustration with yet another hyped technique in drug discovery: structure-based design. While working at the biotech company Hybritech in San Diego in the late 1980s and early 1990s, Stemmer was "disgusted" at the limitations of so-called "rational" design. It yielded "just snapshots", he recalls.

Falling back on his Ph.D. work at the University of Wisconsin, which included some agriculture courses, Stemmer decided to strike out in a new direction. Why not build libraries of proteins in the test tube, then mix and match the genes that coded for these proteins in order to evolve optimal molecules? Such a technique could capitalize on the piles of sequences emerging from the Human Genome Project.

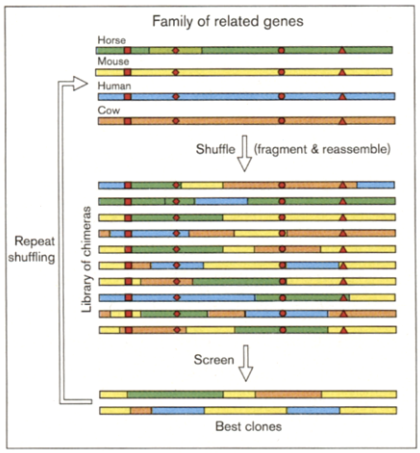
The technique works like this. In the first round, Maxygen begins with the diversity already present in a family of related (homologous) genes. The company then rapidly 'shuffles' all this diversity to create a larger pool of novel genes. Genes are fragmented into small pieces and reassembled based on their original DNA sequences. Then the genes are cloned into bacteria or cells and their protein products are screened for activity. A pool of the best sequences is then simultaneously shuffled and multiplied again using a process based on polymerase chain reaction, then screened again. In a 1994 *Nature* paper, Stemmer showed he had improved the resistance of a bacterium to antibiotics by a factor of 32,000 by shuffling its genes. Conventional techniques of mutagenesis had achieved just a 16-fold improvement.

Winning is the only thing

Stemmer learned early that there would be two keys to making such a library approach successful. First, he would need excellent assays, empirical tests that could determine which individuals in a population of molecules were the 'winners' at some biochemical task. From the first, Stemmer took an utterly pragmatic approach. The better the assays could mimic the task the winners would later be expected to perform, the more suited these winners would be. If he could not think of a sure-fire assay, Stemmer chose a different problem.

Second, Stemmer once again distanced himself from his competitors in making libraries. Many competitors chose to generate huge libraries of millions or even billions of molecules, which were then laboriously sieved. But this approach was like starting "galaxies away" from the eventual successes, says Stemmer, and then working one's way back. Stemmer, by

Figure 1



Family shuffling. Maximizing diversity by recombining related genes. Adapted from an image courtesy of Maxygen.

contrast, started with families of related genes exhibiting some functionality, permutated them and generated further variations on these already productive themes (Figure 1). This has led to a double benefit: the libraries he generates are both smaller and more fertile than those resulting from more random approaches.

Furthermore, because Maxygen chooses the early winners based on their performance in assays rather than due to particular structural or biological features, shuffling departs from what Stemmer calls "knowledge-based approaches" to biotechnology. "We don't have to know how many genes are on a piece of DNA, the sequence of genes or which gene regulates which. We can evolve DNA without any sequence information at all," he says, "just like a child breeding dogs."

Kneeling before nature

This empiricism "makes an academic scientist just cringe," says Ronald Breaker of Yale University in New Haven, a specialist at evolving DNA for many novel purposes. But for Stemmer, remaining empirical is a sign of humility. "Our approach shows respect for the complexity of biological interactions" in strong contrast to the hubris of, say, structure-based design. "The more complex the biology, the more appropriate a breeding approach is," Stemmer adds.

The real power of shuffling has begun to emerge in even more complex proofs of concept. Maxygen has applied the 'family shuffling' technique on 26 variants of the gene for the detergent enzyme subtilisin. These genes were already highly 'engineered', some were patented and the pool included the 'optimal' version of subtilisin already in use in laundry detergents. In a single cycle of shuffling and testing, the team obtained subtilisin 'offspring' that were improved over the parents in three different properties (activity at high pH, thermal stability and solvent stability) simultaneously.

Finally, a Maxygen group recently shuffled genes coding for the billion-US-dollar anticancer and antiviral drug interferon-α. The group shuffled a pool of diverse human genes for interferon-α and, after applying 68 assays, were able to show a 135,000-fold improvement in the molecule's ability to protect mouse cells from infection by a virus.

Growing pains?

All of these successes, and the two recent deals, contribute to a frenetic atmosphere at the company's headquarters at a eucalyptus-scented technology park on the shores of San Francisco Bay. "Before we'd even moved in" in March, observes Maxygen scientist Ling Yuan, "we'd already outgrown our space." The company, which is a now-independent spin-out from the Affymax Research Institute, which in turn belongs to multinational pharmaceutical giant Glaxo Wellcome, has grown from 25 people in early 1998 to a whopping 100 in 1999, with more growth presumably to come.

The company will need all that energy and more to face the dual challenges of applying shuffling to drugs and plants. All of the company's prior work—such as its collaboration with Danish detergent enzyme king Novo Nordisk—has shied away from actual drug discovery. Now the company plans to tackle "more enzymes affecting secondary metabolites," especially in the pharmaceuticals area, where assay development is not so easy, says Jeremy Minshull, a Maxygen group leader in core technologies. It is the company's avowed goal to use shuffling to improve protein pharmaceuticals such as antibodies, growth factors and vaccines, though no corporate deals have yet been announced.

Gene shuffling in plants, too, presents its own perils. On the one hand, agricultural companies are much more aware of the advantages of breeding—"we got the $85 million deal without having published a paper" observes Yuan. At the same time, once the targets reach beyond specific genes in corn or other food plants, Maxygen will need to shuffle entire pathways of genes in order to see an effect. And pathways, says Richard Michelmore, professor of vegetable crops at the University of California at Davis, "are remarkably homeostatic. Tweak one thing and the organism compensates," sometimes in unexpected ways. Still, Michelmore agrees, "engineering whole pathways is where the future is."

Perhaps the biggest hurdle facing Maxygen will be its ability to maintain its high success rate under conditions of rapid growth. Silicon Valley is littered with examples of high-tech companies that grew too fast, resulting in a loss of focus. The company's broader success will depend on how well the company can teach its techniques to its many new employees. There, too, Stemmer is optimistic. After all, if a child can learn to breed, how hard can it be for a Ph.D.?

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